Impact of temperature and defoliation (simulated grazing) on soil respiration of pasture grass (*Cenchrus ciliaris* L.) in a controlled experiment

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Abstract

A controlled experiment was conducted on *Cenchrus ciliaris* L. grass (exotic to Australia) commonly grown in Queensland pastures to investigate the impact of defoliation (simulated grazing), temperature and soil moisture on total soil respiration, and to isolate different components of total soil respiration i.e. the root, root free soil and rhizosphere respiration. The six types of treatments i.e. control (soil only without grass (C1)), control with grass but no defoliation (C2) grown for 9 months, non-defoliated treatments with grass grown for 4 months (D0), and three defoliation treatments (grass defoliated once, D1; twice, D2; and thrice, D3 during growth) were maintained over 9 months. We found that defoliation had no effect on total soil respiration. However, soil temperature accounted for significant changes in total soil respiration across all the defoliation and C2 treatments but not in D0, and the greatest change in soil respiration in response to temperature was noted at the third stage of defoliation, suggesting that defoliation increased the sensitivity of soil respiration to temperature. Root respiration was

significantly ($P < 0.05$) related to root biomass and greater root biomass contributed mainly to increased rate of total soil respiration. The greater sensitivity of total soil respiration to temperature in D1, D2, D3 and C2 treatments and the greater contribution of root respiration in total soil respiration suggests that the root respiration, rather than the total soil respiration, is likely to be more sensitive to change in temperature. With rising ambient temperature and consequently soil temperature, soil CO_2 emissions may increase in a pasture with greater root biomass than that with lesser root biomass.

Keywords: *Cenchrus ciliaris*, defoliation, microbial respiration, root respiration, soil respiration, soil temperature.

Introduction

Soil respiration, a common measure of soil biological activity, represents the amount of CO_2 evolved from roots, soil microbes, and to a lesser extent by oxidation of root exudates, plant detritus and humified organic matter (Raich and Schlesinger 1992). Soils contribute about 10 per cent of total CO_2 emissions to atmosphere (the annual efflux of soil CO_2 is about 76.5 Pg C per yr - Raich and Potter 1995). Moreover, soils are a major sink that store 1500 Pg of C, about twice the amount that is in the atmosphere (Eswaran et al. 1993), thus any factor that affects soil respiration is of major concern for global climate warming (Raich and Tufekcioglu 2000). Among various factors, land use, management practices, and environmental conditions, in particular soil temperature and moisture, are mainly important that influence soil respiration (Raich and Tufekcioglu 2000; Frank et al. 2006).

Globally, each year, forests and savannas alone contribute about 42 Pg of C, while temperate grasslands, tundra, desert, cultivated and other ecosystems contribute only 18 Pg C to the total emissions of about 60 Pg C of respiration (both of vegetation and of microbial decomposition of organic matter) (estimates vary from 60-75 Pg C) (Grace and Rayment 2000). More importantly, soil respiration is about 20 percent greater in grasslands than forest (Raich and Tufekcioglu 2000), and about 1.42 times greater in the tropical grasslands (629 g $C/m^2/yr$) than in the temperate grasslands (442 g $C/m^2/yr$) (Raich and Schlesinger 1992). Therefore, it is vital to understand the soil respiration processes and factors for tropical grasslands.

The present study aims to understand how different components within the soil contribute to total soil CO_2 emissions, and how the management practices and soil temperature and soil moisture affect CO_2 emissions in tropical pastures of central Queensland (Australia). The main objectives are:

- To investigate the impacts of defoliation (simulated grazing), soil temperature and soil moisture on respiration in a sandy soil sown to *Cenchrus ciliaris* L. grass (exotic to Australia) to which land is mostly sown following clearing of trees in Queensland.

- To assess the contribution of root, microbial and rhizosphere respiration to total soil respiration.

The outcomes of this research are useful to support the planning of land use and management policies that promote reduction in soil CO_2 emissions.

Materials and methods

Experimental design and measurements

A pot experiment was conducted in a polyhouse (a shed made of polythene material (mostly green net)) to estimate the contribution of root respiration to total soil respiration, to quantify different components of root, microbial and rhizosphere respiration in soil respiration, and to study the impact of defoliation on soil respiration.

The common pasture grass *C. ciliaris* was grown in pots (34 cm diameter, 35 cm height) to estimate total and root respiration in the absence i.e. non-defoliation and the presence of defoliation i.e. defoliation treatments (simulated grazing - Table 1).

For non-defoliation treatments, plants were grown for 4 months (D0) and terminated by separating roots from soil and then from shoots, to measure root respiration. For defoliation treatments, plants were successively defoliated once (at 4 months; D1), twice (at 4 and 6 months; D2) and thrice (at 4, 6 and 7.5 months; D3) and then terminated by separating roots from soil and then from shoots. Each treatment was replicated five times. Two types of controls were

maintained: C1 with soil but no grass to measure root-free soil (microbial) respiration, and C2 with grass but without defoliation (Table 1).

Ten seeds were sown per pot on 4th April 2002 to a sandy soil, which were later maintained to four clumps per pot. Plants were supplied with hydroponic nutrient solution (Manutec Pty Ltd, SA) during the growth phase. Sandy soil was chosen to minimize root loss during extraction. The pots were watered on average twice a week to maintain soil moisture at 80 % of the field capacity; when the moisture level dropped below 60 % of field capacity pots were watered to 80 % of their field capacity. Soil moisture was monitored with 20 cm Hydrosense probes (CS620: Hydrosense water content sensor, Campbell Scientific Australia).

The experiment was set up in the polyhouse (average temperature 21 ^{o}C (7-32 ^{o}C) and relative humidity 30 per cent (14-48 per cent)); in a randomised block design with four blocks for all treatments. A soil respiration chamber connected to an EGM-3 (Environmental Gas Monitor (EGM-3), PP Systems, UK) was placed on a portion of the exposed soil close to the centre of each pot, along with portable soil moisture and soil temperature probes, for collection of data on soil respiration, soil moisture and soil temperature. These data were monitored from when the plants were two months old (approximate height >30 cm) until their uprooting/termination for root respiration measurements. All the measurements for soil- respiration, temperature and moisture, were taken regularly over 3-5 consecutive days following each irrigation event until the soils were dry (60 % of the field capacity). Similar measurements on control C2 pots continued without any defoliation until the whole experiment was terminated (9 months after sowing). Data collected from the control C2 (grass without defoliation, grown until 9 months) on soil respiration, temperature and moisture were compared with corresponding data taken for defoliation treatments D1 (defoliated once, grown until 6 months), D2 (defoliated twice, grown until 7.5 months) and D3 (defoliated thrice, grown until 9 months) to quantify the effect of

defoliation on total soil respiration. Therefore, for analysis of soil respiration response to temperature, measurements for C2 were divided into 3 sub-sets i.e. data for comparison with D1 were named C2-1, measurements compared with D2 as C2-2, and compared with D3 as C2-3.

To determine the contribution of root respiration to total soil respiration, total soil respiration was recorded for all pots just prior to uprooting or termination. The plants D0 were uprooted after 4 months of growth, whereas D1, D2, D3 and C2, were uprooted after 6, 7.5, 9 and 9 months of growth, respectively. To terminate the experiment, the roots were extracted by emptying a pot onto a plastic sheet to remove the plants from the sand. Shoot parts were then removed by cutting and sand was removed by gentle shaking, before measuring the root respiration. A special PVC chamber, the same diameter as that of the soil respiration chamber, was constructed. The roots were placed in the PVC chamber and the soil respiration chamber was placed vertically on this to measure respiration (R_{root}). All the pots were uprooted, one by one, with measurements completed within a minimum time gap (of 2-4 minutes) and without letting the roots dry. All the procedure to uproot plants from pot, to remove roots and to measure root respiration took only up to 2-4 minutes, and it was assumed that this short time would not disturb root respiration. Excising roots to measure root respiration has been commonly used given the time period is not very long (Kocyigit and Rice (2006) reported that a time period of <30 minutes did not affect root respiration). After respiration measurements, roots were washed to remove the sand particles (if any), dried at 60 ^{o}C for 48 hours to measure root biomass.

The pots were refilled with the same soil once the plants were removed, and monitored at 3-4 day intervals until respiration stabilised (normally two months after uprooting the plants). The stabilised rate of respiration was taken as root-free soil respiration/microbial respiration (R_{rfs}) as per the basal respiration method outlined by Kelting et al. (1998). R_{rfs} was then used to estimate

rhizosphere respiration (R_{rhizo}) (i.e. respiration attributable to microbial activities and decomposition of root exudates in the rhizosphere), as follows (Kelting et al. 1998):

Total soil respiration $R_s = R_{root} + R_{rfs} + R_{rhizo}$ (i)

Where each component is measured as:

R_s (total soil respiration) - using soil respiration chamber

R_{root} (root respiration) - by root extraction using the special PVC soil respiration chamber

R_{rfs} (root free soil respiration) - using the basal method (stable respiration from the empty soil pots after removing the grass roots).

Rearranging equation (i) solving for R_{rhizo}:

R_{rhizo} (rhizosphere respiration) = $R_s - (R_{rfs} + R_{root})$ (ii)

The above-mentioned procedure, a combination of two methods i.e. the root extraction and the basal, was followed to determine root, microbial and rhizosphere respiration and to avoid overestimation of any of these three components of soil respiration (details mentioned in Sangha et al. 2003). Use of one method alone, either the extraction or basal method, overestimates the root and microbial components of total respiration (Kelting et al. 1998). These are explained below:

Excising roots by extraction to measure R_{root} overestimates R_{rfs} (microbial respiration) since rhizosphere respiration is not isolated: $R_{rfs} = R_s - R_{root}$ (R_{rfs} includes rhizosphere respiration).

The basal method measures R_s and R_{rfs}, and then R_{root} is calculated as: $R_{root} = R_s - R_{rfs}$, but it overestimates root respiration since the rhizosphere component is not isolated (R_{root} includes

rhizosphere respiration). A related approach to estimate root and rhizosphere respiration of wheat was also applied by Kocyigit and Rice (2006).

Statistical analysis

The soil, root, microbial/root-free soil, and rhizosphere respiration, and root biomass data were analysed for ANOVA to compare various treatments, using Genstat ver 6.0 (2002). Simple regression analysis was used for the relationship between R_{root} and root biomass. The response of R_s to temperature and moisture was analysed using exponential regression separately for all treatments.

Results

The rate of total soil respiration (R_s) and root respiration (R_{root}), expressed as g CO_2 m^{-2} soil surface h^{-1}, increased over the period of growth from four to nine months. The R_s of defoliation treatments D1, D2 and D3 did not differ from their corresponding non-defoliated controls C2 (C2-1 against D1, C2-2 against D2 and C2-3 for D3); defoliation *per se*, therefore, had no effect on R_s (Table 2).

Defoliation has no effect on the rates of R_{rfs} (root free soil/microbial) respiration and R_{root} per unit root biomass (Table 2). However, the rate of rhizosphere respiration (R_{rhizo}) was greatest in D2, followed by D3 and D1 and was the least in D0 (no defoliation - Table 2).

R_{root} increased with plant growth (Table 2), and was unaffected by defoliation as apparent from the comparison of rates between R_{root} in non-defoliated C2 and defoliated D3 treatments. R_{root} was closely ($P < 0.05$) related to root biomass (Fig 1). The increased rate of R_{root} with the growth of plants was entirely due to the increase of biomass, as consequently was the increase in R_s.

Among the factors affecting R_s, soil temperature accounted for significant variation in R_s (r^2= 0.64 at *P<0.05*) across all defoliation treatments D1, D2, D3 and their control C2 (Fig 2 and Table 3). The response of R_s to change in temperature was very small in C1 (control without plants; r^2 = 0.07 at *P<0.05*) for a temperature range of 16.6 ^oC-25.4 ^oC and in D0 (4 months growth but no defoliation; r^2 =0.13 at *P<0.05*) for a temperature range of 10.8 ^oC-25.9 ^oC. The response of R_s to change in temperature was greater in treatments where plant growth was > 4 months (D1, D2, D3 and C2). The temperature quotient $Q_{10,}$ calculated for all the measurements taken during the experiment, was 3.31 for temperature range 10.8 ^oC-25.9 ^oC (based upon exponential regression analysis for y (soil respiration) = $0.0387e^{0.1197x}$ (where x is temperature, taken as 10 ^oC)).

Among defoliation treatments, the response of R_s to temperature in D3 differed significantly from that in its control C2-3 (Fig 2 and Table 3). However, D1 and D2, and their respective controls C2-1, and C2-2 did not differ significantly among themselves.

Soil moisture accounted for only four per cent of the variation in soil respiration in all the treatments (Fig 3), possibly due to smaller range in soil moisture (60-80 % of the field capacity) during this experiment. Therefore, temperature proved to be a main factor that conditioned variation in soil respiration in this experiment.

Discussion

Most research on soil respiration has focused on temperate climates (e.g. Boone et al. 1998; Bowden et al. 1993; Giardiana and Ryan 2000; Valentini et al. 2000), with little relevance to tropical climates. The present study on R_s was conducted in a tropical climate including, in part both winter and summer seasons, with the additional factor of defoliation (simulated grazing). This study suggested that grazing *per se* did not influence R_s (since the rates were similar in

defoliation treatments and their respective non-defoliated controls), however, the root respiration increased with plant growth, the later was also reported by Kocyigit and Rice (2006) for wheat. On average, roots contributed about 50% of R_s (Table 2). Raich and Tufekcioglu (2000) reported 10-40% contribution of R_{root} to total soil respiration in temperate pastures, and Kocyigit and Rice (2006) reported on average 35 % R_{root} contribution (for a range of 20-65 %, during different stages of growth in wheat (grown in controlled conditions)). The greater contribution of R_{root} to R_s in the present study could be due to congenial conditions for grass growth and hence the root biomass. This suggests that for tropical pastures, the grass species should be selected carefully where roots lead to minimal soil CO_2 emissions while having efficient up take of water and nutrient for plant growth and pasture yield.

Defoliation had no effect on microbial or root respiration, but did influence rhizosphere respiration (R_{rhizo}) at D2 stage only. However, the reasons for increase in R_{rhizo} in D2 and then decline in D3 compared to D2 are not very clear. Manske (2000) reported that defoliation promotes the growth of soil microorganisms at certain stage of plant growth; this could be a reason for increase in R_{rhizo} at D2.

The sensitivity of R_s to temperature in the present experiment was in line with studies from temperate regions (Kicklighter et al. 1994; Valentini et al. 2000). The Q_{10} value of 3.31 for measurements over winter and summer seasons of tropical climate of central Queensland was similar to the average Q_{10} = 3.1 calculated by Kicklighter et al. (1994) based upon data from various temperate studies. Valentini et al. (2000) suggested that the temperate zones are more sensitive to increase in soil respiration with increase in temperature. In the present experiment, soil temperature varied from 10 ^oC to 26 ^oC, as pots were set in a polyhouse (made of polythene materials, without controlled conditions). These variations were less compared to the outside temperatures, an average minimum and maximum temperatures of 6-8 ^oC and 23-25 ^oC during

winter (June-August), and 22-24 ^{o}C and 33-36 ^{o}C during summer (December-February). Moreover, pots were well watered and shaded, thus had different climate compared to the outside climatic conditions, and the pastures in the field where summer is quite hot and dry. It is likely that the Q_{10} value may be different for field measurements where wide range in soil temperature and soil moisture over a year can notably affect R_s.

The change in R_s in response to temperature in defoliation treatments D1, D2 and D3, and non-defoliation control C2 compared to D0 and C0 (only 4 months old plants in D0 and no plants in C0) suggested that plant growth, most likely the increase in root biomass, was the main reason for such an increase in R_s. Thus, over time with plant growth, pastures may contribute more to R_s. Boone et al. (1998) reported that root respiration, rather than the total soil respiration, is more sensitive to temperature. Since the increase in R_s was highly related to root biomass, hence the pastures with greater root biomass would probably contribute more to R_s, and such pastures will be more sensitive for CO_2 emissions to change with temperature. If there is an increase in temperature in global climate, as predicted by IPCC (2005), then soil CO_2 emissions will most likely increase in pastures with greater root biomass.

The significant impact of temperature on R_s in D3 compared to its control C2-3 suggests that defoliation increased R_s sensitivity to temperature (Fig 2) since the root biomass did not differ significantly between D3 and C2-3. The increase in R_s in D3 was, therefore, mainly attributable to temperature. This suggests that after a certain amount of plant growth, defoliation may increase the sensitivity of R_s to temperature. If so, then in exotic pastures where grazing is carried over repeatedly, increased root respiration from increase in root biomass with plant growth over time, and the increased sensitivity of R_s to temperature (as apparent from greater sensitivity in D3 compared to its non-defoliated control C2-3) can result in higher soil CO_2 emissions. Therefore, the total soil CO_2 emissions may increase with repeated grazing over time

firstly due to increase in root biomass and hence respiration, and secondly due to sensitivity of R_s to change with temperature.

Soil moisture was not an important factor for R_s, mainly because it was maintained between 60-80 % of field capacity. Soil moisture may have a significant impact under extreme conditions of dry and moist for longer duration, as noted over the range 4 to 17 % prior to and following an in-field rainfall event in a grazing system of central Queensland (Kaur et al. 2006).

The methods used to isolate different components of soil respiration, as proposed by Kelting et al. (1998) are simple and easy to apply, and lead to interpretable results. A similar approach was also applied by Kocyigit and Rice (2006) to isolate root and rhizo-microbial respiration from total soil respiration in wheat plants. The authors suggested that excising roots to measure respiration did not affect root respiration given the time period to excise and measure respiration is short (<35 mins). For the present study, measuring root respiration following excised method took much less time (<4 minutes), and allowed to isolate microbial and rhizosphere components of total soil respiration. Other advanced methods such as isotope techniques (details of various methods discussed by Hanson et al. 2000) exist but they are costly.

Data from this experiment have emphasised the contribution of R_{root} respiration to $R_{s,}$ and the sensitivity of R_s to temperature for pastures in tropical climates, and suggests that land use practices i.e. type of grass, grazing period and spelling (frequency and interval) can influence soil CO_2 emissions. *C. ciliaris* generally comprises 1:1 root: shoot ratio (Peverill et al. 1999; and the same was observed in the present experiment (data not presented)) and its roots contribute about 50 % of R_s. A study on native or other pasture species that may have a higher shoot to root ratio, and that contribute less to soil CO_2 emissions, is called for. Understanding the contribution of different components of, and the impacts of soil temperature and soil moisture on total soil

respiration for various plants could be useful to select plants that emit less CO_2 but have good pasture yield, and can help to plan land use policies to reduce soil CO_2 emissions in tropical pastures.

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Table 1. Various treatments to estimate different components of soil respiration.

	Treatment	Abbreviations used in text	No. of pots	Growth period (months)
Control 1	No grass, soil only	C1	5	-
Control 2	No defoliation	C2	5	9
I set	No defoliation	D0	5	4
II set	Defoliation - once	D1	5	6
	Defoliation - twice	D2	5	7.5
	Defoliation - thrice	D3	5	9

Table 2.Total soil (R_s), microbial (root-free soil) (R_{rfs}), rhizosphere (R_{rhizo}) and root (R_{root}) respiration (g $CO_2/m^2/hr$) per soil respiration chamber (volume 1885.71 cm^3), and root respiration (R_{root}) per unit root biomass (g), and root biomass (RB) (g) per pot for controls C1 (soil but with no grass) and C2 (with grass, no defoliation) after 6, 7.5 and 9 month growth (R_s for all, but $R_{rfs,}$ R_{rhizo} and R_{root} measured only after 9 months when uprooted), and for plants before (D0) and after successive stages of defoliation (D1-once, D2-twice and D3-thrice) treatments.

Treatments –controls and defoliation (growth period)	R_s*	R_{rfs}*	R_{rhizo}*	R_{root}*	R_{root} /RB*	RB/pot*
C1-soil only	0.02^{d}	0.02^{a}				
C2-no defoliation:						
C2-1 (6 month)	0.58^{b}					
C2-2 (7.5 month)	1.02^{a}					
C2-3 (9 month)#	1.13^{a}	0.02^{a}	0.39^{b}	0.72^{a}	0.07^{a}	129.4^{a}
D0-no defoliation (4 month)	0.19^{c}	0.01^{a}	0.08^{c}	0.09^{c}	0.08^{a}	11.1^{b}
D1-defoliated once (6 month)	0.76^{b}	0.01^{a}	0.38^{b}	0.37^{b}	0.09^{a}	41.3^{b}
D2-defoliated twice (7.5 month)	1.37^{a}	0.02^{a}	0.93^{a}	0.42^{b}	0.07^{a}	69.0^{b}
D3-defoliated thrice (9 month)	1.22^{a}	0.02^{a}	0.31^{b}	0.89^{a}	0.08^{a}	121.2^{a}
LSD	*0.268*	*0.025*	*0.178*	*0.200*	*0.02*	*41.96*

* Different superscripts in a column represent significant difference according to least significant difference of means (LSD) at $P = 0.05$

C2 plants were uprooted once only (after 9 months)

Table 3. Regression analyses for exponential relationships ($R_s = a + b\ e^{(-k*temperature)}$) between soil respiration and soil temperature for various treatments.

Treatment	a (Intercept)	b (Slope)[#]	k	r^2 *
D1 (Defoliated once)	0.4065	0.00001749	-0.1806	0.64
D2 (Defoliated twice)	0.6278			
D3 (Defoliated thrice)	0.8055			
C2-1 (Control against D1)	0.3711			
C2-2 (Control against D2)	0.7421			
C2-3 (Control against D3)	0.7584			
D0 (no defoliation)	n.s.			
C0 (Control with no plant)	n.s.			

[#] Slopes were not significantly (at *P<0.05*) different across various treatments hence all data had a common slope, meaning that the lines were parallel but with different intercepts.

* for pooled data from defoliation treatments D1, D2, D3 and their controls C2-1, C2-2 and C2-3.

n.s. - no significant response of soil respiration to soil temperature at *P<0.05*.

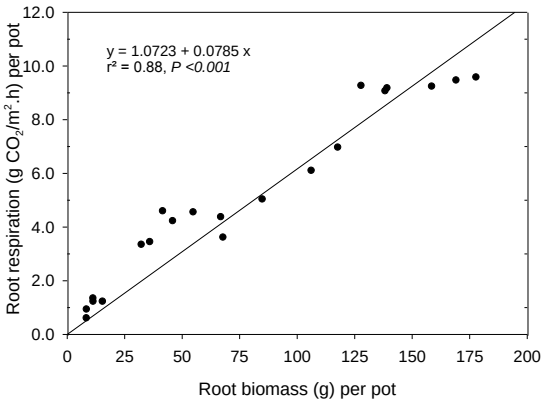

Fig. 1. Relationship between root respiration and root biomass for *C. ciliaris.*

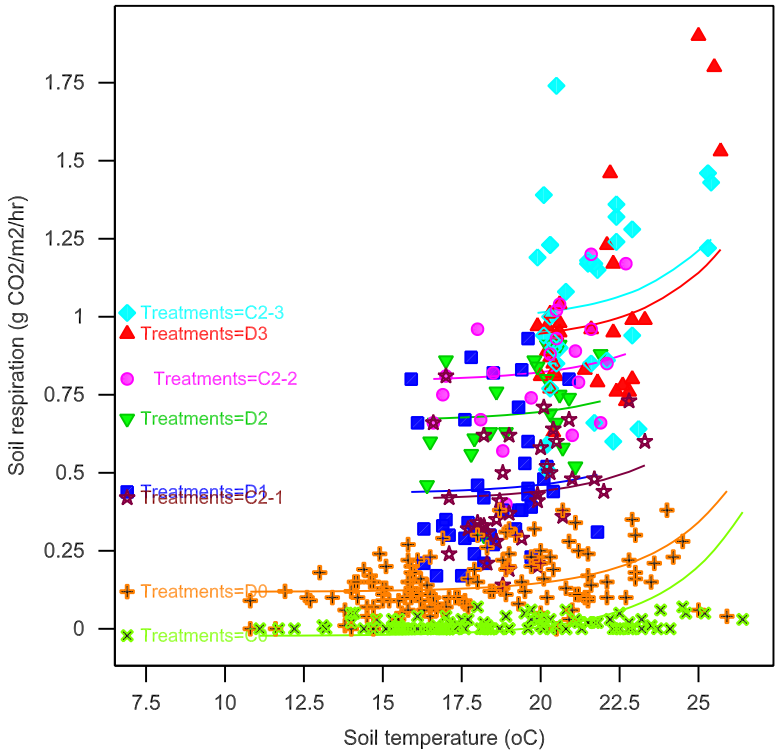

Fig 2. Soil respiration response to soil temperature for defoliation treatments and their corresponding controls (graph reproduced from Genstat) (corresponds to Table 3):
D1 (defoliated once) and control C2-1 (treatment labels are overlapping in plot)
D2 (defoliated twice) and control C2-2
D3 (defoliated thrice) and control C2-3
D0 (no defoliation)
C0 (control with no grass) (treatment labels C0 and D0 are overlapping in plot)

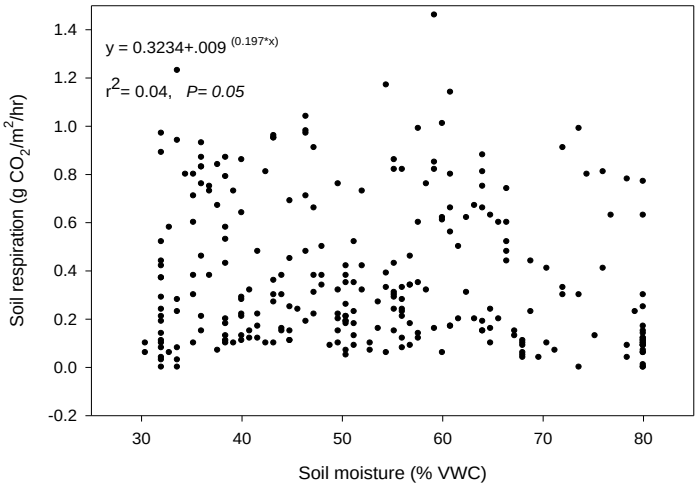

Fig 3. Soil respiration response to soil moisture (percentage volumetric water content) in all treatments (C1, C2, D0 and defoliation treatments D1, D2 and D3).